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Studies on Allelochemicals in *Synedrella nodiflora* and Impact of Its Leaf Leachates on Germination and Seedling Growth of Radish (*Raphanus sativus*) and Mustard (*Brassica juncea*)

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Synedrella nodiflora is the highly dominant obnoxious invasive alien weed species through out the campus of Pune University, India. Its leaf leachates stimulated the seed germination of mustard (*Brassica juncea*) and radish (*Raphanus sativus*) at low concentrations (2.5-5.0 %), but higher concentrations (15-20 %) were most inhibitory to both test plants. The higher concentrations were most inhibitory to seed germination and seedling growth. The phytochemical and TLC analyses revealed that *Synedrella* leaves were rich in phenolics and various neutral and non-polar compounds and allelochemicals like tannins and glycosides.

Key Words: Allelochemicals, Allelopathy, Bioassay, *Brassica juncea*, Germination, *Raphanus sativus*, *Synedrella nodiflora*.

INTRODUCTION

At present weed scientists are developing integrated weed management strategies for their long term management[1], as they cause greater losses (up to 45 %) in crop yields, than the agricultural pests[2]. Nowadays, the crop fields and forests are invaded by many exotic weeds like *Cassia, Synedrella, Parthenium, Chromolaena, Lantana, etc.* which have posed a serious problem[3]. These invasive alien species greatly influence the plant biodiversity in natural ecosystem and replace the native species[4]. Despite several allelopathic studies on these plants, their mechanism of invasion is not fully understood[5].

Synedrella nodiflora (L.) Gaertn. (family Asteraceae) originated in tropical South America is an annual, herb and is now distributed all over India[6]. Its dominance is due to wide adaptability, deep roots, faster growth

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rate, most effective and efficient pollination and seed formation ability, seed polymorphism and its effective dispersal methods, biotic and abiotic stress tolerance along with synthesis of some novel allelochemicals.

Pandya[7] claimed that detailed allelopathic research on weed-weed and weed-crop interactions is lacking in India. Previous studies[8,9] revealed that plant biodiversity has been reduced by invasive alien weed species like *Alternanthera, Cassia, Synedrella, Parthenium* and *Acalypha* in the campus of Pune University. Hence to explore the mechanism of invasion of exotic weed *Synedrella* in Pune University campus, the present study was initiated. The allelopathic potential of invasive weed *Synedrella nodiflora* was investigated using seed germination bioassay technique on sensitive crops like mustard and radish.

EXPERIMENTAL

The leaves of *Synedrella* were collected during the flowering stage cleaned with distilled water and spread on filter paper for shade drying. The dry leaves were grounded in Wiley Mill to pass two mm sieve. 100 g powdered leaves were soaked in 1000 mL distilled water for 24 h at 25 ºC and the leachate was filtered through Buchner funnel. It was stored in refrigerator in amber coloured bottle (to avoid degradation).

Seed germination bioassay: The seeds of radish (*Raphanus sativus*) and mustard (*Brassica juncea*) were obtained from College of Agriculture, Pune. Healthy seeds were used for bioassay study in seed germination chamber using sterilized petri plates (9 cm dia) lined with germination papers. The seeds of radish and mustard were surface sterilized with 0.02 % aqueous $HgCl_2$ for 2 min. Then the seeds were thoroughly washed with distilled water. Seed germination papers were thoroughly moistened (5 mL) with respective concentrations of leaf leachates of *Synedrella* (2.5-20.0 % v/v) which were prepared by dilutions with distilled water. The seeds kept in distilled water were considered as control. 20 Seeds of radish and 40 seeds of mustard were uniformly kept in each petri plate. 2 mL Leachates of respective concentration were added in each petri plate on third day. The petri plates were irrigated with the leachates only once. The treatments were replicated thrice. Seed germination (%), root and shoot length, total seedling height and vigour index[10] was recorded on 7th day.

Extraction, isolation and identification of allelochemicals: Shade dried powdered leaf material of *Synedrella* (5 g) was refluxed with ethanol for 12 h. The leachate was filtered and residual solvent was completely dried under reduced pressure. To detect phenols and neutral compounds[11], precoated aluminium TLC plates were used. These were developed with 10 % ethanol in toluene as solvent system and were observed under UV lamp at 365 nm. The five different pink chromophoric glowing spots were

marked and then developed by iodine crystals to determine the various compounds.

Statistical analysis: The data were analyzed statistically using ANOVA test.

RESULTS AND DISCUSSION

Mustard: It has been observed that leaf leachates of *Synedrella* inhibited seed germination and seedling growth in mustard at all the concentrations used. The percentage of inhibition was concentration dependent (Table-1). These results have shown the sensitivity of mustard to the leaf leachates. The LC_{50} value in mustard was obtained at 11.25 % concentration of leaf leachates, which is in between 10 and 12.5 % concentrations. These findings are in agreement with other researchers[12-14].

TABLE-1
EFFECTS OF *Synedrella nodiflora* LEAF LEACHATES ON SEED GERMINATION OF MUSTARD

Leaf leachates conc. (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index	Root:shoot ratio
Control	97.50	4.36	4.39	481.00	0.88
2.5	87.50	3.60	5.16	452.08	0.69
5.0	82.50	2.66	5.10	420.75	0.52
7.5	75.00	1.83	4.56	342.50	0.39
10.0*	70.00	1.53	2.83	198.33	0.55
12.5*	32.50	0.80	1.80	58.50	0.44
15.0	27.50	0.53	2.20	60.50	0.24
17.5	0	0	0	0	0
20.0	0	0	0	0	0
CD at 1 %		0.406	0.954	74.716	0.135

*Mean LC_{50} value.

The 2.5 % concentration of *Synedrella* leaf leachates enhanced the shoot length, but the root length and root:shoot ratio decreased over other control. The higher concentrations however have shown inhibitory trend in all the parameters. The phytotoxic effects of various weeds on seedling germination and growth of treated plants are well reported[15,16]. The allelopathic impact of leachates or extracts is usually more harmful to radicle[17] as it comes in close contact with them. The phytotoxicity was directly proportional to concentrations of leachates[18,19]. The results of present studies are in agreement with different researchers[20,21]. Vigour index also was affected in mustard at all concentrations. It indicates the allelopathic effects on seedling establishment[22].

Radish: At 2.5 % leaf leachate concentration of *Synedrella* showed the seed germination at par with that of control, however seedling growth

was decreased. The magnitude of reduction in all the parameters increased with the concentrations of leachates. LC_{50} value (17.5 %) indicated higher tolerance of radish to allelochemicals in *Synedrella* leaf leachates (Table-2). Similar phytotoxic effects of *Parthenium* residues were observed on radish and chickpea[23]. The weeds *Avena*, *Cyperus*, *Polygonum* and native weeds like *Solanum* caused similar effects on wheat and frenchbean[24-26]. The leaf leachates significantly decreased the vigour index in radish as compared to control.

TABLE-2
EFFECTS OF *Synedrella nodiflora* LEAF LEACHATES ON SEED GERMINATION OF RADISH

Leaf leachates conc. (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index	Root:shoot ratio
Control	100.00	3.60	3.80	380.00	0.94
2.5	100.00	2.10	2.50	250.00	0.83
5.0	90.00	1.80	2.30	207.00	0.78
7.5	90.00	1.80	2.83	255.00	0.63
10.0	70.00	1.50	2.43	170.33	0.61
12.5	65.00	0.50	2.00	110.00	0.27
15.0	60.00	1.10	2.10	126.00	0.51
17.5*	50.00	0.86	1.66	83.33	0.51
20.0	15.00	0.40	1.43	21.50	0.28
CD at 1%		0.919	0.741	85.126	0.174

*LC_{50} value.

Allelochemicals in *Synedrella* leaves: The allelochemicals present in *Synedrella* leaf leachates revealed that they had inhibitory impact on root and shoot growths. Similar results have been reported by many workers[27-30]. In cold solvent extraction method *Synedrella* leaves showed the presence of non-polar, semi-polar and polar components (Table-3). When plant material was kept in contact with various solvents at room temperature, easily soluble components (as per their polarity) released in the solvents. The hexane extracted material contained more non-polar matter (15.8 %) while chloroform extracted material was only about 0.3 % *i.e.* very less and acetone extracted value was highest about 22 %.

The maximum extractions of compounds were indicated by semi-polar (22 %) followed by non-polar (15.8 %) and minimum in polar solvents (11.8 % MeOH, 11.1 % EtOH) (Table-3). The TLC showed presence of as major components, as one non-polar component, three semi-polar components and six polar components. The common allelochemicals detected were starch, proteins, sugars, *etc.* (Tables 4 and 5).

TABLE-3
COLD SOLVENT EXTRACTIVE VALUES OF
Synedrella nodiflora LEAVES

Solvents	Extraction (%)	No. of spots	UV absorption at 365 nm		Major components	TLC plate no.
			No. of spots	Colour		
Haxane	15.8	2	-	–	1	1
Chloroform	0.3	5	3	Pink, Pink, Orange	2	1
Ethylacetate	8.0	3	1	Pink	1	1
Acetone	22.0	2	-	–	-	1
Ethanol	11.1	5	3	Fluorescent, Green, Violet.	3	2
Methanol	11.8	5	3	Green, Pink, Violet.	3	2

TABLE-4
QUALITATIVE DETECTION OF
ALLELOCHEMICALS IN *Synedrella* LEAVES

Test	Method	Observation	Inference
Starch	Material + conc. H_2SO_4	Dark brown colour	Presence
Proteins	Material + conc. HNO_3	Orange colour	Presence
Tannins	Material + $FeCl_3$	Greenish yellow colour	Presence
Steroids	Material + Acetic acid	Green colour	Presence
Sugar	Material + Benedict solution	Green colour	Presence

TABLE-5
QUALITATIVE DETECTION OF
ALLELOCHEMICALS IN *Synedrella* LEAVES

	Cold solvent extraction					
Test	*n*-Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol
Starch	Absence	Absence	Absence	Absence	Absence	Absence
Proteins	Absence	Absence	Presence	Presence	Presence	Absence
Tannins	Presence	Presence	Absence	Presence	Presence	Presence
Steroids	Absence	Presence	Presence	Presence	Presence	Presence
Sugar	Presence	Absence	Presence	Presence	Presence	Presence

The solvents like methanol, ethanol, acetone and ethyl acetate showed the presence of allelochemicals proteins, carbohydrates, tannins and glycosides. Both the solvents like chloroform and hexane showed presence of tannins in common whereas glycosides and carbohydrates in particular. The leaf leachate of *Synedrella* contained maximum phenolics (0.85 %) followed by neutral compounds (0.47 %) and acids and bases in negligible quantity (Table-6).

TABLE-6
QUANTITATIVE ESTIMATION OF
ALLELOCHEMICALS IN *Synedrella* LEAVES

Chemical group	Observed (%)
Acid	Negligible
Phenol	0.85
Base	Negligible
Neutral	0.47

The leachates of *Synedrella* were containing five different phenolic compounds out of which four were fluorescent (Table-7). Amongst these, two were major ones as compared to remaining fluorescent compounds. Similarly, the leachates of *Synedrella* showed presence of seven different types of neutral compounds out of which only five were major ones. Amongst these, three compounds were showing absorbance at 365 nm. These allelochemicals from semi-polar and phenol group compounds may be responsible for inhibition of seed germination[31-33]. Similar results were obtained with other invasive weeds like *Chromolaena*[34] and *Cucurbita* species[35].

TABLE-7
RESULT FOR TLC'S OF PHENOL AND NEUTRAL EXTRACT

Types	No. of spots on TLC	UV absorption at 365 nm		Major component	TLC plate no.
		No. of spots	Colour		
Phenol	5	4	–	2	Plate no. 3
Neutral	7	3	Pink, Green, Green	5	Plate no. 4

Thus, the detection of allelochemicals in *Synedrella* leachates may help in explaining its invasiveness over native weed species. Present investigations may be useful for ecofriendly management of *Synedrella* and may also help to understand the weed-weed and weed-crop interactions in agro-ecosystems or forest ecosystems. The potential novel biomolecules (allelochemicals) present in the leaves of *Synedrella* may be exploited for the management of weeds, insects, diseases and nematodes in sustainable agriculture after further studies.

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